ENHANCEMENT OF GIBBERELLIN GROWTH-PROMOTING ACTIVITY BY HYDRANGENOL ISOLATED FROM LEAVES OF HYDRANGEA MACROPHYLLA [1]

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Hydrangenol (4′, 8-dihydroxy 3,4-dihydro isocoumarin)[3] was first isolated from the flowers of *Hydrangea hortensia* Smith, by Asahina and Miyake (1). Recently the authors isolated the aglycone and glucoside of hydrangenol from leaves of Engel's White hydrangeas. Coumarin and other unsaturated lactones are known to inhibit growth and germination (6, 14), but apparently there are no published accounts of the effect of the closely related isocoumarins on growth. The experiments reported here deal with the effect of hydrangenol in enhancing the growth-promoting activity of gibberellin.

Methods and Results

Characterization of Isolates From Engel's White Hydrangea Leaves: Two kg of fresh Engel's White hydrangea leaves from dormant plants stored at 4 to 5° C for 4 weeks were extracted by grinding with 80 % ethanol in a blendor. The aqueous ethanol extract was filtered through Hyflo Super-Cel and reduced to the aqueous phase by distillation in a vacuum. Chlorophyll was removed by filtration and the aqueous phase was extracted with ethyl ether in a liquid-liquid extractor for 2 weeks. After 48 hours of continuous extraction hydrangenol crystals were visible. Hydrangenol was removed from the ethyl ether and recrystallized three times from hot water. The yield was 2.4 g of the aglycone and 0.6 g of the glucoside of hydrangenol. The recrystallized hydrangenol was all converted to the aglycone by hydrolyzing with 2 N hydrochloric acid at 100° C under reflux for 1 hour.

The isolates from leaves of Engel's White hydrangeas were found to be indistinguishable from the glucoside and aglycone of authentic hydrangenol[4] (table I). Elemental analysis showed that the aglycone contained 70.24 % C, 4.67 % H, and 25.09 % O, whereas the theoretical values for $C_{15}H_{12}O_4$ are 70.28 % C, 4.72 % H, and 25.00 % O. The isolated glucoside yielded only glucose upon hydrolysis. The

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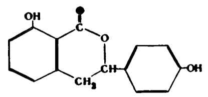

[4] The authors are indebted to R. K. Ibrahim, Botany Department, McGill University, Montreal 2, Canada, for a sample of authentic glucoside and aglycone of hydrangenol.

Table I

R_f Values for Authentic Hydrangenol (4′8-dihydroxy 3,4-dihydro isocoumarin) and Isolates from Leaves of Engel's White Hydrangeas

Solvent	R_f Values			
	Authentic hydrangenol		Isolates from leaves of Engel's White hydrangeas	
	Aglycone	Glucoside	Aglycone	Glucoside
n-Butyl alcohol: benzene: ammonium hydroxide (50:2:48 v/v)	0.59	0.15	0.58	0.15
n-Butyl alcohol: acetic acid: water (6:1:2 v/v)	0.96	0.71	0.96	0.71
n-Propyl alcohol: ammonium hydroxide (7:3 v/v)	0.85	0.47	0.85	0.46

isolated aglycone and glucoside both melted at 179° C (uncorrected), a value slightly lower than 181° to 182° C reported by Asahina and Miyake (1).

The ultraviolet absorption spectra of the isolates and the authentic aglycone and glucoside of hydrangenol were identical. In 95 % ethanol the aglycone and glucoside had absorption maxima of 315 and 300 mμ, respectively.

The cumulative evidence of paper chromatography, elemental analysis, melting point, and ultraviolet absorption spectra indicates that the compounds isolated from leaves of Engel's White hydrangeas are identical with the aglycone and glucoside of hydrangenol.

The aglycone of hydrangenol was used to study plant responses. This compound is sparingly soluble in water but soluble in dilute sodium hydroxide. A stock solution for treating plants was made by dissolving 1 mg of the aglycone of hydrangenol per 1 ml of hot water and adjusting the pH to 7 with sodium hydroxide. The final volume was adjusted to contain 0.1 % Tween-20.

ELONGATION OF MAIZE SHEATH: Seeds of d-1 mutant dwarf maize[5] were grown by the method described by Neely and Phinney (12). Seeds previously soaked in water for 24 hours were planted in soil in flats and then placed in a greenhouse with a minimum night temperature of 30° C. Approximately one week after planting, normal seedlings were removed and the dwarf ones just beginning to develop the first leaf were treated as shown in table II. Ten μl of the aglycone of hydrangenol and 10 μl of gibberellic acid (A_3), in appropriate concentrations, were placed separately at the base of the first sheath. The second leaf sheaths were measured to the nearest millimeter when they were fully expanded (12 days).

The aglycone of hydrangenol applied alone had little or no effect on the elongation of the second leaf sheath of d-1 mutant dwarf maize (table II). Elongation of the second leaf sheaths of d-1 mutant dwarf maize increased with increased concentrations of gibberellic acid applied to the plants. The response to 0.5 μg of gibberellic acid was increased by adding the aglycone of hydrangenol. Elongation of the second leaf sheath of d-1 mutant dwarf maize treated with a higher concentration of gibberellic acid (5 μg/plant) was increased by applying 0.1 μg of the aglycone of hydrangenol, but higher concentrations of the aglycone of hydrangenol tended to reduce the growth promotion of gibberellic acid.

In the previous test the solutions were applied to the same area of the first leaf sheath. To determine if the activity depended upon applying the chemicals to the same or separate areas, the aglycone of hydrangenol and gibberellic acid were applied from a mutual solution to the same area and from separate solutions to separate areas of the first leaf sheath of d-1 mutant dwarf maize. The total concentration of the surfactant applied to the plants was always constant. As shown in table III the aglycone of hydrangenol enhanced the growth-promoting activity of gibberellic acid to the same degree regardless of method of application.

[5] Seed supplied through the courtesy of B. O. Phinney, Department of Botany, University of California at Los Angeles.

TABLE II

MEAN LENGTH (mm) OF FULLY EXPANDED 2ND LEAF SHEATH OF d-1 MUTANT DWARF MAIZE TREATED WITH GIBBERELLIC ACID AND AGLYCONE OF HYDRANGENOL*

GIBBERELLIC ACID (μG/PLANT)	HYDRANGENOL AGLYCONE (μG/PLANT)				
	0.0	0.1	1.0	10.0	50.0
0.0	14	12	10	16	18
0.5	26	44	44	46	39
5.0	83	101	76	79	74

LSD 5 % = 14.7. 1 % = 22.8.
* Average for five plants per treatment.

TABLE III

MEAN LENGTH (mm) OF FULLY EXPANDED 2ND LEAF SHEATHS OF d-1 MUTANT MAIZE TREATED WITH GIBBERELLIC ACID AND AGLYCONE OF HYDRANGENOL ON SEPARATE AREAS FROM SEPARATE SOLUTIONS OR ON SAME AREA FROM A MUTUAL SOLUTION*

GIBBERELLIC ACID (μG/PLANT)	SOLUTION	HYDRANGENOL AGLYCONE (μG/PLANT)			
		0	0.01	0.1	1.0
0	...	22	25	25	21
0.1	Separate	40	49	49	46
0.1	Mutual	37	47	52	44

LSD 5 % = 4.6. 1 % = 7.3.
* Average for ten plants per treatment.

ELONGATION OF PEA AND BEAN: Seeds of *Pisum sativum* L. cultivar Meteor and *Phaseolus vulgaris* L. cultivar Black Valentine were soaked in water for 24 hours, planted in soil in 3-inch clay pots, and placed in a greenhouse at a minimum night temperature of 25° C. Approximately one week after planting, seedlings of uniform height were selected for treatment. Ten μl of the aglycone of hydrangenol and 10 μl of gibberellic acid in appropriate concentrations were placed separately on the leaves surrounding the growing point. The aglycone of hydrangenol was applied after the gibberellic acid solution was dry. Seedlings were measured to the nearest millimeter 1 week after treatment.

Gibberellic acid accelerated elongation of both Meteor pea and Black Valentine beans in relation to concentration. Gibberellic acid applied at the rate of 0.1 μg promoted extension of the second and third internodes of Meteor pea from 3.7 to 8.2 cm and Black

TABLE IV

MEAN LENGTH (cm) OF 2ND AND 3RD INTERNODES OF METEOR PEA 7 DAYS AFTER TREATMENT WITH GIBBERELLINS AND AGLYCONE OF HYDRANGENOL*

Gibberellins		Hydrangenol aglycone (μg/plant)			
Type	(μg/plant)	0.0	0.1	1.0	10.0
None	0.0	5.6	4.9	4.8	4.6
A_1	1.0	11.6	16.2	14.9	10.9
A_2	1.0	7.2	7.5	8.3	7.3
A_3	1.0	21.4	25.9	22.4	22.0
A_4	1.0	8.5	9.1	9.5	10.0

LSD 5 % = 2.4. 1 % = 3.8.
* Average for five plants per treatment.

Valentine bean from 9.2 to 16.3 cm. Applying 0.01 μg of the aglycone of hydrangenol plus 0.1 μg of gibberellic acid further increased the extension to 10.9 cm for the former and 22.3 cm for the latter[6]. Growth promotion by higher concentrations of gibberellic acid was not affected by applying the aglycone of hydrangenol.

GROWTH RESPONSES TO FOUR TYPES OF GIBBERELLIN AND AGLYCONE OF HYDRANGENOL: Seeds of Meteor peas were grown and the plants treated as previously described. Solutions of four pure gibberellins[7] (A_1, A_2, A_3, A_4) were prepared from warm distilled water containing 0.1 % Tween-20. Maximum growth responses of Meteor peas were obtained with A_3 followed in order by A_1, A_4, and A_2 (table IV). They are in agreement with the results reported by Bukovac and Wittwer (5).

Adding 0.1 μg per plant of the aglycone of hydrangenol significantly increased growth-promotion by gibberellin A_1 and A_3. Similar results were obtained with elongation of the second lateral peduncle of Shasta chrysanthemums.

DRY WEIGHT OF HYDRANGEA: Plants of *Hydrangea macrophylla* Ser. cultivar Sainte Therese were grown in an air-conditioned greenhouse at a mean temperature of approximately 22 to 25° C. The maximum temperature rarely exceeded 30°. Plants were treated weekly from June 4 until September 18 by spraying 1 ml of a solution containing 1 μg gibberellic acid and 1 μg of the aglycone of hydrangenol to the terminals of each plant. Plants were harvested September 24 and dried at 80° in a forced-draft oven. The values shown in table V indicate that applying the 16 μg of gibberellin resulted in significantly increased plant growth as reflected by stem extension and deposition of solids. The aglycone of hydrangenol alone exerted little effect but when combined with gibberellic acid it tended to enhance accumulation of total solids in the leaves and stems.

DISCUSSION

The action of gibberellin in promoting stem elongation and accumulation of total solids is well established (4, 7, 10, 17, 18). The present experiments show that a naturally occurring material, hydrangenol, increases the growth-promoting activity of low levels of exogenous gibberellin when applied to several test species. The material had little or no growth-stimulating activity alone and it failed to enhance the activity of higher levels of gibberellin. These results suggest that the aglycone of hydrangenol in some way affected the absorption or metabolism of gibberellin. Since the aglycone of hydrangenol increased the growth-promoting activity of gibberellic acid to the same degree whether applied from separate solutions to separate areas or from a mutual solution to the same area of the first leaf sheath of d-1 mutant dwarf maize, it must be affecting the cellular metabolism of the plant rather than enhancing the absorption of gibberellic acid.

Most plants have been postulated to contain naturally occurring gibberellins (16). Bean (15) and citrus (9) have been shown to contain A_1. Gibberellic acid, A_3, has not been isolated in a crystalline form from flowering plants. As shown the aglycone of hydrangenol alone was inactive but enhanced the growth-promoting activity of applied A_3 and A_1 much more than it did of the other two gibberellins. These results indicate that the aglycone of hydrangenol promoted primarily the activity of applied gibberellin, but did not affect the endogenous gibberellin.

The stem extension results suggest that the aglycone of hydrangenol is similar in action to the third factor postulated by Brian and Brian and Hemming (2, 3) and further expanded by Galston and Warburg (8) but interpreted by them as a sparing action of

TABLE V

GROWTH OF SAINTE THERESE HYDRANGEA PLANTS TREATED WEEKLY (JUNE 4 UNTIL SEPTEMBER 18) WITH 1 μG PER PLANT PER WEEK OF GIBBERELLIC ACID AND AGLYCONE OF HYDRANGENOL*

Treatment	Plant height cm	Mean dry wt/plant: Stems g	Leaves g	Total g
Control-untreated	18.2	3.0	16.9	19.9
Hydrangenol aglycone	18.5	3.1	16.1	19.2
Gibberellic acid	36.6	5.2	19.1	24.3
Gibberellic acid + hydrangenol aglycone	36.7	5.8	21.2	27.0
LSD 5 % =	4.1	1.0	3.0	3.7
1 % =	5.5	1.3	4.0	5.0

* Average for ten plants per treatment.

[6] LSD at 5 %. Meteor peas = 1.4, Black Valentine bean = 2.2.

[7] Supplied through the courtesy of Yusuke Sumiki, Department of Agriculture Chemistry, Faculty of Agriculture, University of Tokyo, Tokyo, Japan.

auxin. The possibility must be considered that the aglycone of hydrangenol in some way exerted an auxin-sparing action which resulted in the increased growth. If this mechanism be accepted then one should expect that A: Application of the aglycone of hydrangenol alone should have enhanced the activity of gibberellins occurring naturally in the test plants and B: larger amounts of the aglycone of hydrangenol alone should have enhanced the activity of higher levels of applied gibberellin unless auxin became limiting. Neither of these actions was observed. However, Neumann (13), has reported that coumarin and several derivatives promoted the extension of small sections of Helianthus hypocotyls. Supra-optimal concentrations initially promoted extension but subsequently inhibited growth. Mayer (11) demonstrated that coumarin inhibited the action of gibberellin in promoting the germination of lettuce seeds. Both of these experiments and those which suggested the third factor were conducted with excised or isolated parts of the plant. Normal synthesis, translocation, and utilization of compounds was limited. This report deals with the intact plant where the complications of continuing growth preclude any simple explanation of the mechanism by which the aglycone of hydrangenol enhances the growth-promoting activity of applied gibberellin.

Summary

The aglycone and glucoside of hydrangenol (4′8-dihydroxy 3,4-dihydro isocoumarin) were isolated from leaves of Engel's White hydrangeas. The aglycone of hydrangenol by itself possessed little or no biological activity but when applied to several test species it enhanced the activity of low concentrations of applied gibberellin.

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